

## REMARKS

This Amendment is submitted preliminary to the issuance of an Office Action in the present application and in response to the Official Action of July 30, 2009.

In the last Office Action, the Examiner has rejected claims 4, 6-8, 15, 21-24 and 34-37. Claims 13, 14, 16-20 and 26 to 33 are withdrawn from further consideration and are hereby cancelled. No claims were added. In view of the Examiner's rejection claims 4, 34 and 35 have been amended.

Applicant contends that claims 34 and 35 as currently amended overcomes the rejection based on US Patent No.: 6,130,101 to Mao ("Mao"). Amended claim 34 and 35 now specify that the Cyc1 is substituted at the ortho-position of the ring attached to the backbone of formula (I) with  $-\text{CONR}_5\text{R}_6$ . Support for the amendment is found in paragraph [0050] of the specification. In addition, this can also be seen from Examples 1-30 and 37-60.

According to one aspect of the present invention, lacton- or lactam forming dyes having different functional groups can be modified in a simple manner such that they do not become colorless through lacton or lactam formation, and instead can be used as markers in the described manner in many areas. See also, paragraph [0005] and [0043] of the specification.

Based on the preferred ortho-positioned  $-\text{COOH}$  substituted compounds (II), ortho-substituted compounds of formula (I) result from the conversion with secondary amines  $\text{HNR}_5\text{R}_6$  where  $\text{R}_5$  and  $\text{R}_6$  are hydrocarbons. Due to the  $\text{CONR}_5\text{R}_6$  group, a lactam-forming reaction is no longer possible, thus realizing compounds that are suitable as markers for a variety of applications.

On the other hand, compounds having the drawbacks of the type as discussed in paragraph [0005] of the specification and those as taught by the cited prior art of Mao are not suitable as markers.

The rhodamine dyes that are taught in Mao show carboxamide function, where the carboxamide groups are positioned in meta- or para-position; see also Examples 25-31 in Mao. In all such examples, the carboxamide groups are

derived from primary amines  $-NHR$  and thus have the tendency for lactam formation. Clearly an object to be avoided by the present invention. Therefore the Mao reference does not render the claimed compounds obvious.

Due to the cost-efficient and thus desirable production of rhodamine dyes from phthalic acid anhydride and aminophenolen, all rhodamine dyes contain an ortho- carboxyphenyl-sustituent in the center of the chromophore system. The ortho-COOH group has however a drawback decisive against many applications: depending upon the degree of protonization, absorption and fluorescence shift up to 10 nm, and in an unpolarized environment (such as for example a cell membrane etc.) lacton can form resulting in the disappearance of the typical dye absorption and fluorescence.

Another drawback of such dyes is that the  $-COOH$  groups cannot be used for covalently labeling of analytes, as an immediate lactam formation results from the coupling with a primary amine group (e.g. from lysine), which then no longer has any dye properties and thus becomes unsuitable for labeling of an analyte.

By means of the presently claimed invention all drawbacks of rhodamines having a free  $-COOH$  group are avoided. By converting the  $-COOH$  group into a secondary amide, absorption and fluorescence become independent of pH; and since lactam formation is no longer possible, dye absorption and fluorescence remain intact. They also remain also independent of polarity. It is also possible to modify one of the residuals  $R_5$  and  $R_6$  with a functional group, for example a carboxyl group. Such a group is incapable of forming a lactam or lacton due to the large distance to the chromophore, thereby permitting coupling to a biomolecule or similar without any problems.

The Mao examples on the other hand show even in the ortho-position a  $-COOH$  or a  $-COO$  group, which leads to the above described drawbacks, and render absorption and fluorescence pH dependent as well as polarity dependent. The non-sensitivity as mentioned in Mao in col. 2, lines 34-36 relative to pH changes do however not relate to examples 25-31.

For the forgoing reasons independent claims 34 and 35 are not rendered obvious by the Mao reference.

As for the rejection of the retained dependent claims, these claims depend directly or indirectly from claim 34, share its presumably allowable features, and therefore it is respectfully submitted that these claims should also be allowed.

Withdrawal of the rejection of claims 4, 6-8, 15, 21-24 and 34-37 under 35 U.S.C. §103(a) and allowance thereof are thus respectfully requested.

### CONCLUSION

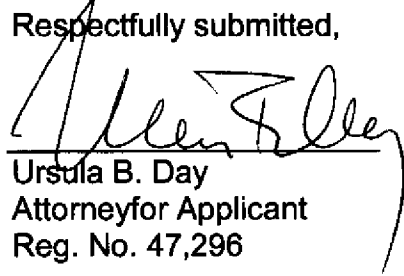
In view of the above, each of the presently pending claims in this application is considered patentably differentiated over the prior art of record and believed to be in condition for allowance. Reconsideration and allowance of the present application are thus respectfully requested.

Should the Examiner consider necessary or desirable any formal changes anywhere in the specification, claims and/or drawing, then it is respectfully requested that such changes be made by Examiner's Amendment, if the Examiner feels this would facilitate passage of the case to issuance. If the Examiner feels that it might be helpful in advancing this case by calling the undersigned, applicant would greatly appreciate such a telephone interview.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1747.

Respectfully submitted,

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